SELECTION OF FOOD IN STENTOR CAERULEUS (EHR.)

BY

ASA ARTHUR SCHAEFFER

A DISSERTATION SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

SUBMITTED MAY 15, 1909

SELECTION OF FOOD IN STENTOR CAERULEUS (EHR.)

BY

ASA ARTHUR SCHAEFFER

A DISSERTATION SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

SUBMITTED MAY 15, 1909

BIOGRAPHY

Asa Arthur Schaeffer is of Pennsylvania-German and French ancestry, and is the son of Peter P. Schaeffer and Lucinda Schaeffer, née Engler. He was born May 9, 1883, at Kunkletown, Pa. His preliminary education was obtained in the public schools of that place, and in Polytechnic Institute, at Gilbert, Pa., from which latter place he was graduated in June, 1900. In September of the same year he entered Franklin and Marshall College. In June, 1904, he was graduated from this institution with the degree of A. B. In October, 1904, he entered Johns Hopkins University, enrolling as a graduate student in zoölogy, and taking physiology and botany as subordinates. The first two years were spent in absolving the requirements of the subordinate subjects and of other preliminary work. During the scholastic year 1906-1907 he was absent from the University. In October, 1907, he again entered the University, and from that time to date has worked upon selection of food in protozoa, in the Zoölogical Laboratory of the Johns Hopkins University, under the direction of Professor Jennings.

In the year 1907–1908 the subject of this sketch held a University Scholarship, and during the year 1908–1909 he held a University Fellowship. He is at present, during the summer months. on the teaching staff in the Biological Laboratory at Cold Spring Harbor, Long Island, N. Y.

ACKNOWLEDGMENTS

I wish here to express my gratitude to Prof. H. S. Jennings for his generous and most valuable assistance in, and criticism of, my work while at Johns Hopkins University.

To Professors Howell, Johnson, and Andrews, and to all the other members of the Biological and Physiological Laboratories, and to Professor Watson of the Psychological Laboratory, I also express my sincerest thanks for their valuable aid and kindness in various matters.

This being my first opportunity, I wish also to indicate here my deepest appreciation of the treasured teachings of the late Professor Brooks, under whose guidance I studied for my first two years at Johns Hopkins. His philosophical discussions especially appealed to me, and it is chiefly because of the pleasure derived from listening to Professor Brooks' enquiries into the philosophical aspects of biological problems, that I feel impelled to add this little note of sincerest appreciation.

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41548215_0055

SELECTION OF FOOD IN STENTOR CÆRULEUS (EHR.)[1]

BY

ASA ARTHUR SCHAEFFER

WITH TWO FIGURES

Introductory 75
Material 78
Food of Stentors 79
Methods of investigation 80
Normal behavior of Stentor 81
Behavior in rejecting particles 84
Experiments with specific particles 88
Experiment 1 Discrimination between Phacus and sulphur 88
Experiment 2 Discrimination between starch grains and Phacus 90
Experiment 3 Discrimination between starch, glass, and Phacus 92
Experiment 4 Selection of Phacus and Euglena from sulphur and glass 93
Experiment 5 Discrimination between Phacus triquetur and P. longicaudus 96
Experiment 6 Discrimination between different species of organisms 96
Experiment 7 Discrimination between different species of organisms 96
Experiment 8 Discrimination between organisms of similar size and shape 101
Experiment 9 Effect of hunger on behavior in feeding 104
Experiment 10 Effect of satiety on behavior in feeding 104
Experiment 11 The effect of utter satiety on Stentor 109
Experiments with mixtures of particles 111
The basis of selection 121
The selective mechanism 125
Summary 126
References 128

INTRODUCTORY

The question whether protozoa can exercise selection in the kind of material which they feed upon has called forth expression of opinions from almost every worker upon the protozoa. The inges-

[1] From the Laboratory of Experimental Zoölogy, Johns Hopkins University. The author of this paper is indebted to Prof. H. S. Jennings under whose direction this investigation was carried on.

tion of insoluble material is readily observed in many of these organisms, and this fact together with the generally accepted notion that the protozoa are much simpler organisms than the recent work shows them to be, was perhaps largely responsible for thinking the selection of food a question answerable by more or less casual observation and by inference from the general behavior. This is brought out by comparing the views of some of the more important workers.

Prior to Verworn's work, which may be regarded as the point of departure for the recent interest in the protozoa, the consensus of opinion of workers in this field seems to have declared in favor of the ability of these organisms to ingest certain kinds of particles and to reject certain other kinds in a systematic manner. Thus Stein ('67) and Entz ('88) and others declared unequivocally that in infusoria, whenever "foreign" particles were brought by the "alimentary vortex" into the "pharynx," the current was stopped or given such a direction that the foreign particle was swept away. Neither of these writers, however, seems to have taken into account nor tried to explain the earlier observations of Ehrenberg ('38) and others, who described the ingestion by certain infusoria of large quantities of carmine grains, which can hardly be regarded as anything but "foreign" particles.

Verworn ('89) took up this question and confirmed Ehrenberg's results with carmine; he also observed that chalk crystals, indigo particles, and the like, were freely ingested. At the same time that these indigestible particles were swallowed, small organisms such as swarm spores and micrococci were often swept away by the cilla. From these and other observations Verworn concluded that there is no selection obtaining among the various kinds of particles which the alimentary vortex brings to the mouth of the infusorian.

Bütschli ('89) also came to the conclusion that the power of choice of food is absent in the protozoa.

But in 1893 the opposite view was again advanced by Hodge and Aikins, who said that "a prine condition of the creature's (Vorticella) life must be its ability to distinguish food from what is not food." But there is no reference to or explanation of either

Ehrenberg's or Verworn's work mentioned above, where indigestible particles were described as being freely eaten.

Jennings in 1902 worked upon Vorticella and confirmed Ehrenberg's and Verworn's experiments; he showed that Hodge and Aikins' conclusions were probably drawn from insufficient data. Similar experiments upon Stentor also showed that this infusorian ingested large quantities of carmine, india ink, etc. From these experiments Jennings came to the conclusion that these organisms probably do not have the power of selecting their food in any precise way.

In 1907 there appeared a preliminary paper by Metalnikow "Ueber die Ernährung der Infusorien und deren Fähigkeit ihre Nahrung zu Wählen," in which the author describes the taking up or ingesting of carmine and india ink by paramecium, but states that if left in water in which is suspended carmine or ink, the paramecia gradually take in less and less of these substances until in about 18 days few or none contain either ink or carmine. According to Metalnikow the paramecia are gradually "educated" in some way so that they cease after awhile to take the carmine or ink. This paper will be more fully discussed further on.

This brief historical account includes most of the more important references to the ability of protozoa to select food. These references are all incidental in character and the experiments upon which they were based were in almost every case few and not varied. This lack of experimentation was probably due to the notion that if they could discriminate at all, the protozoa should tell with precision for each and every particle whether it is food or not, and that any mistakes would be sufficient evidence that the ability to choose among particles of various sorts is absent. In short, machine-like accuracy seems to have been expected if selection is present at all.

But we can hardly with clear thinking demand more prefect selective faculties in the protozoa than in the higher vertebrates. If one should draw the conclusion that because one observes a horse eat bits of a weather-beaten fence rail, the horse has not the ability to select his food, the logic would be equivalent to that which is used when it is affirmed that because a protozoan eats car-

mine, the protozoan cannot express choice in food. The phrase "selection of food" is evidently vague, and great care is necessary in interpreting results when this concept is applied to specific instances. In this paper the words "selection," "choice," etc., are used only in a purely objective sense.

These considerations, together with the fact that most protozoa are observed to contain in their bodies only food materials in various stages of digestion, has led the present writer to carry out a number of experiments on this matter, using many substances, digestible as well as indigestible. Because of large size, transparency, sessile habit, and highly developed ciliary apparatus, the Blue Stentor (Stentor cæruleus Ehr.) was selected as affording probably the best opportunity for investigation. The question proposed, was: Does Stentor ingest all particles that reach its disk, or are swept into its pouch; or are some eaten and some rejected, depending on whether they are or are not good for food? This is the central question in this paper. A number of other matters were also dealt with, as: the existence of conditions of hunger and satiety; the basis of the selection, whether chemical or tactual, etc. These questions will be taken up at their proper places.

MATERIAL

The Stentors used in these experiments and the organisms used for food were collected from a number of widely separated localities, viz: Cold Spring Harbor, L. I.; Kunkletown, Pa., and various places around Baltimore. This was done to determine whether there were differences in Stentors that grew in different localities with regard to the power of selecting food. After it was found that Stentors from all localities and from laboratory cultures gave practically the same results, the larger part of the work was done upon specimens raised in the laboratory. All the organisms used for food were also raised in the laboratory, excepting Phacus and Euglena, which were always collected from wild cultures. The food of the Stentors raised in the laboratory consisted of bacteria and paramecia almost exclusively. Many of the Stentors which were used in the experiments where Euglenæ and Phacus were fed,

had not eaten any of these latter organisms prior to the experiment, nor had their ancestors for many generations eaten either Phacus or Euglenæ.

FOOD OF STENTORS

It is of course not always easy to determine what materials actually serve as food and what do not; this is particularly difficult in so minute an animal as Stentor. The application of tests for food value used with higher animals is quite impracticable. deciding whether certain things should be classed as food for Stentor, the following criteria were employed: (1) Long continued feeding of the substance in question must not injure the animal in any way. (2) The material must decrease in quantity in passing through the body, showing that some part has been absorbed. These two tests were applied with great care to the substances which Stentor was seen to eat. Tabulated results follow.

1 Substances eaten rather freely which do not serve as food:

Powdered carmine	Powdered india ink	Powdered charcoal
Dead yeast plants (?)	Raphidium	

2 Substances eaten only occasionally which do not serve as food:

Powdered glass	Fine sand	Powdered sulphur
Potato starch grains	Bits of detritus	

3 Substances eaten freely which serve as food:

Small Stentors	Paramecia	Chlamydomonas
Phacus triqueter	Phacus longicaudus	Euglena viridis
Euglena spirogyra	Euglena deses	Trachelomonas hispida
Trachelomonas volvocina	Stylonychia	Monostyla
Arcella	Coscinodiscus	Lyngbya
Oscillaria	Peranema	Chilomonas
Hydatina	Colpidium	Amœba
Halteria	Spirostomum	Bacteria

METHODS OF INVESTIGATION

There are two methods by means of which choice of food can be investigated in such an organism as Stentor.

One method is specific, consisting in observing and recording the path and fate of each particle that is fed to the Stentor. This is accomplished as follows: A capillary pipette is made by drawing out an ordinary pipette to a very fine hair having an internal diameter of about 75μ or less. Food particles, such as Phacus, Euglenæ, etc., or indigestible particles, as the experiment may demand, are then sucked up into the pipette with some water. Several Stentors are transferred from the original culture dish into a watch glass with a few cubic centimeters of the culture solution and placed on the stage of a binocular microscope of the Braus-Drüenr type. A magnification of about 65 diameters is used. After the Stentors have become attached to bits of detritus in the watch glass, the particles are fed from the pipette, the end of which is held very carefully about the diameter of the disk away from and above the Stentor's disk. The particles are for the most part fed successively, in each case waiting until the foregoing particle is swallowed before the succeeding one is set free from the pipette. Much time is of course taken up in the recording of results and in getting the pipette into position again for feeding. Some idea of the time required in making such feeding experiments may be obtained from the fact that a successful experiment in which 120 particles are fed extends over about an hour and three-quarters. When it was desired to feed two or more kinds of food, the particles were first mixed in the desired proportion and then sucked into the pipette. If the various sorts did not come in the desired order some of the particles were merely dropped to the bottom of the dish and not allowed to touch the disk of the Stentor at all. In this way the order of substances in a mixed stream was under control. Nothing was fed when it was not intended. With a method of this degree of exactness there seems to be no reason why the results should not be thoroughly reliable. The only considerable variant which seems possible is the physiologic state of Stentor—which it is the purpose of this paper to investigate.

While the method outlined above is the best possible for solving many of the questions that are bound up with that of choice of food, there are nevertheless other points which cannot well be cleared up in this way. For it is well known that Stentors eat and thrive upon such small organisms as bacteria, and it would be impossible for several reasons to note what happens to each individual bacterium as it is swept into the Stentor's pouch. For getting at questions of this nature there was employed another method which may be called an indirect method as compared with the one described above.

When it is desired to test the relative readiness with which very small particles such as bacteria, Peranema, yeast cells, finely ground carmine, etc., are taken up by the Stentor, it has been found that the best method is to mix up quantities of them in the desired proportion and then to introduce into this mixture some normal Stentors which have very little or no food in them. After a stated time the Stentors are taken out and squeezed under a cover glass in order to examine their contents. The most difficult point is to maintain in the mixturue the original uniform distribution of the particles. This difficulty was mostly overcome by frequent stirrings and by placing the dish in the dark to prevent reactions to light, as will be described more fully later. By the use of this method very important results have been reached which could not have been obtained otherwise. Each of these two methods acts as a check upon the other, and at the same time they verify each other's results.

THE NORMAL BEHAVIOR OF STENTOR

To understand the experiments bearing on the choice of food the reader should have a more or less clear idea of the normal behavior of Stentor. This subject has been dealt with to some extent by various observers, notably by Jennings, but in the course of my investigations several points have been cleared up that were heretofore more or less imperfectly understood. Several new features of behavior have also been discovered that play at certain stages essential rôles in the choice of food, and it is necessary

perhaps that these should be fully described at this point to avoid the necessity of giving an account of them while discussing the experiments.

In a normal attached Stentor in a watch glass under a binocular microscope there are observed four groups or systems of cilia by means of which the greater part of the behavior is effected. (See

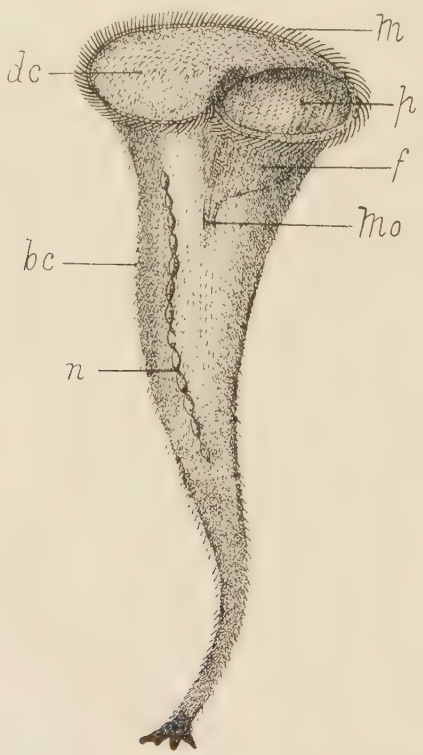

Fig. 1. Normally extended "hungry" Stentor. *b c*, Body cilia; *d c*, discal cilia; *f*, funnel; *m*, membranellæ; *mo*, mouth; *n*, nucleus; *p*, pouch.

Fig. 1.) These four systems are: (1) the membranellæ, (2) the discal cilia, (3) the cilia of the pouch and funnel, and (4) the general body cilia found on the sides of the Stentor. Each of these sets of cilia has a function quite different from that of any of the other groups, and the extent to which their behavior can be modified also differs among the various groups.

The most conspicuous of these ciliary appendages are of course the membranellæ, which are inserted around the rim of the disk. Their normal action creates the well known alimentary vortex by means of which a constant stream of water is caused to flow against the disk. In this manner the Stentor procures whatever small particles there may happen to be suspended in the water. The particles as well as the water in which they are suspended are driven against the disk more or less perpendicularly. The water is driven out over every part of the rim of the disk, while the particles striking against the disk are carried slowly by the discal cilia toward the pouch. The transportation of the particles on the disk is not due to any feature of the movement of the membranellæ, but is entirely due to the discal cilia. These are very small organs and are disposed in rows more or less parallel to the membranellæ. Their action cannot be observed except under the high power of a compound microscope. Their arrangement, size, etc., can then also be seen. The particles, as they strike the disk, are taken by these cilia and slowly passed on in the direction of the pouch, over the rim of which the particles are dropped. The particles are then taken by the pouch and funnel cilia and either passed down to the mouth, where they are ingested, or else are swept out over the rim of the pouch on the ventral side of the Stentor. The cilia of the pouch and funnel are considered as of one system because their functions are identical as far as can be determined. It has been found impossible to observe just how these cilia beat under various conditions. It is of course quite certain that when a particle is ingested they beat downward toward the mouth, and that when a particle is rejected they beat in the opposite direction. It is also found that a small particle in the center of the pouch is little acted upon by the cilia, but as it comes nearer the cilia it begins to travel faster. It is probable therefore that the transfer of particles in the pouch and funnel is effected more by actual contact with the cilia than by mere transportation in a current of water which is set in motion by their action, and this is probably true also for the action of the discal cilia. Only small particles are wholly transferred by the action of the pouch and funnel cilia. Large particles, such as paramecia, are ingested

by the combined action of the cilia and the compressive movement of the walls, of the pouch and funnel. The general body cilia play the least important part in the securing of food. They are distributed in rows over the surface of the Stentor included between the foot and the edge of the disk. Their chief function is that of locomotion. But when the Stentor is attached, their backward or footward beat helps to get rid of the water flowing over the edge of the disk which no longer contains food, and also carries out of reach of the vortex those particles which are dropped over the edge of the pouch in the mid-ventral notch. By this means the vortex always consists of water and particles which for the most part had not struck the disk before. The body cilia thus serve to increase the food-getting ability of the Stentor. This constitutes the normal action of all the cilia of a normal attached Stentor under usual circumstances when ingesting food.

BEHAVIOR IN REJECTING PARTICLES

The rejection of a particle is accomplished by various modifications of the ciliary movements outlined above, depending upon the strength of the stimulation. In the simplest case the rejection of a particle is produced by a reversal of the cilia in the pouch, or in the funnel, or in both. There is much variation even in this apparently simple method of getting rid of an objectionable particle. The course of the particle may be altered at any stage in passing from the interior surface of the pouch to the mouth opening at the bottom of the funnel. If the substance is a small sand grain, e. g., the course is in almost every case altered before the funnel opening is reached. That is, the sand grain is ejected by a strong, presumably outward, beat of the pouch cilia. The funnel cilia play no part in such an instance, as may be seen occasionally when there is a food particle in the funnel at the time there is a sand grain in the pouch. The sand grain is ejected while the food particles in the funnel are ingested. When the substance is one like a starch grain, or a grain of carmine, or a food particle when the Stentor is not "hungry," the course of the particle may be altered anywhere from the interior surface of the pouch to the mouth open-

ing. It may go down to the mouth and be nevertheless finally rejected, or it may go only half way down before its direction is reversed. And further, the particle may travel back and forth many times in the funnel, or in the pouch, or through the extent of both, before either ingestion or rejection takes place. For the sake of shortening the description in the subsequent experiments this traveling back and forth of a particle will be described as the forming of "loops" in its path, each reversal from the ultimate direction (as determined by the fate of the particle) being considered as one loop. Such loops in the path of a particle in the funnel often take place while another particle in the pouch is either rejected or passed on into the funnel. These two sets of cilia may therefore beat quite independently of each other.

Other methods of rejecting or getting rid of substances are also frequently employed especially when there are large numbers of objectionable particles impinging on the Stentor's disk, as clouds of carmine or other indigestible material. In addition to a reversal of the membranellæ, bending away, contraction, and breaking of the foothold and swimming away, which reactions have been described by Jennings ('02) there are several other methods of reacting toward large quantities of indigestible particles which have not heretofore been described. Some Stentors close up the rim of the pouch almost completely for longer or shorter periods when surrounded by dense clouds of carmine. This method of preventing the ingestion of particles of carmine is most frequently observed after the Stentor has torn away from its foothold and is swimming freely in the water. This method is undoubtedly effective but for some reason the reaction is not persisted in for any length of time. Another and much more interesting modification of behavior occurs also under conditions similar to those which induce closure of the pouch, but it is observed in Stentors which have become attached again though still surrounded by dense clouds of carmine. Stentors under these circumstances are not quite as fully extended as when carmine is absent, but all the groups of cilia function as usual except with this interesting modification at times. The discal cilia instead of carrying the particles on toward the pouch as they strike the disk, roll the particles

around on the aboral side of the disk, in "push ball" fashion, in a rather large circle in the direction from right to left along the dorsal edge. (See Fig. 2.) Since the particles are not got rid of as soon as they strike the disk they accumulate in large, loose

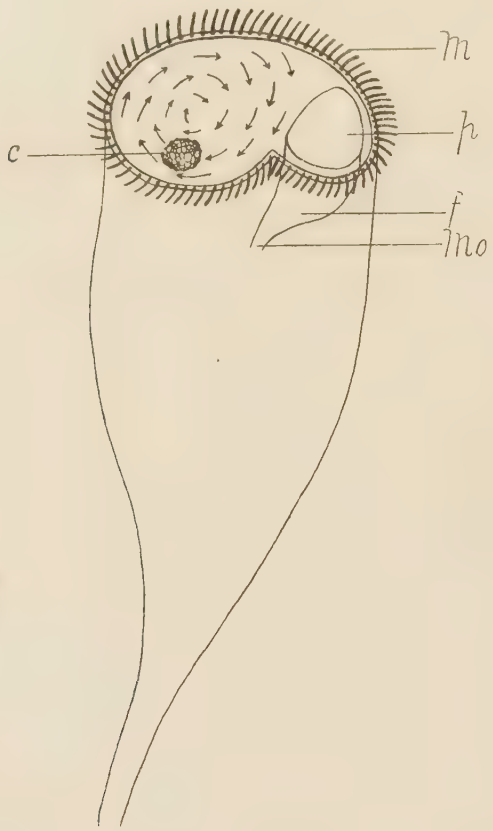

Fig. 2. Illustrating the "push ball" method of getting rid of objectionable particles. The arrows show the direction of beat of the discal cilia. *c*, mass of carmine grains; *f*, funnel: *m*, membranellæ; *mo.*, mouth; *p*, pouch.

masses which are, after some time, either by a special reaction or by accident—I didn't determine which—dropped over the edge of the disk, opposite the pouch. The carmine masses move slowly and it can be readily seen that the motion is altogether due to the action of the discal cilia. This method of preventing the ingestion

of indigestible particles is very effective. Very few particles ever get into the pouch so long as this mode of ciliary action takes place. This modification of behavior appears gradually and also disappears gradually; and during the transitions the action is imperfect, some particles passing into the pouch while others are carried to the right before dropping into the pouch. This phenomenon is interesting in that the discal cilia, which act usually in a certain definite coördinated way, are able to change their behavior so as to act in an entirely different but still coördinated way. What is more remarkable still perhaps is that some of the cilia beat in the same direction in both cases, while some others beat in an exactly opposite direction, the rest of them beating in every conceivable direction between their usual direction of beat and its direct opposite. This is a very good example of the extreme plasticity of behavior of such an organism as Stentor.

Under the same conditions in which the foregoing change of behavior was observed there was found another method by means of which the Stentor made its ingesting apparatus ineffective. This was done by contracting and staying contracted. Contractions for more than several minutes have not heretofore been recorded, but in one set of Stentors I observed continuous contraction for more than two and three-quarter hours. There was not a single relaxation during all this time and it is possible that this state of continuous contraction lasted longer than two and three-quarter hours, for the observation was not continued until relaxation occurred. The body cilia remained constantly reversed in this case, but the membranellæ frequently alternated between the usual and the reversed beat. Both groups of these cilia beat less vigorously than when relaxed or free swimming. The pouch and funnel were closed and no particles whatever were ingested.

Still other Stentors under similar circumstances differed in behavior from all the above. Under stimulation of dense clouds of carmine some Stentors swam with the foot ahead continuously for over three hours. The membranellæ were sometimes beat in the ordinary and sometimes in the reversed way, but always with a less vigorous beat than when the Stentor is fully extended attached, and in water with few particles present. There was no

spiral turning or revolving on the long axis. The Stentors swam in a circle as one would expect from a consideration of the crescentic shape of the partly extended Stentor. This method of behavior also resulted in the ingestion of but very few particles.

These are probably the chief constituents of what may be called the normal behavior of Stentor. We shall next consider the experiments which were designed to answer the question: Can Stentor discriminate between food and indigestible particles?

EXPERIMENTS WITH SPECIFIC PARTICLES

We have just seen that the normal behavior of Stentor is very complex for an organism of such simple anatomy, especially as far as the movements and action of the cilia are concerned. We saw that there are at least four distinct groups of cilia—five, if the pouch and funnel cilia are considered as two groups—each of which has a more or less definite thing to do under ordinary circumstances; but the moment that conditions obtain which are unusual, the behavior of one or more of these systems of cilia changes. We saw that any one of these four or five groups of cilia can change its behavior while the rest of the ciliary apparatus beat in the usual manner; or all the cilia of the entire Stentor can change direction and force of beat upon occasion so that entirely changed behavior results. In fact with these four or five groups of cilia which may beat independently of each other and vary their behavior in different ways, it seems hardly possible that a situation could confront a Stentor which could not satisfactorily be met by having recourse to the many possibilities of the varying behavior of these ciliary systems. The next problem then was to devise an experiment that should test the efficiency of this highly adaptive ciliary apparatus in discriminating between food and indigestible particles.

Experiment I. Discrimination between Phacus and Sulphur

For this purpose roll sulphur was ground up into a fine powder and then thoroughly stirred with a large quantity of water. After

the coarser particles had settled down the finer particles were siphoned off to be used in the experiment. The water in which the sulphur was stirred up was filtered water from the Stentor culture, so that the results of the experiment cannot be attributed to any peculiar qualities of the water. Some of the particles of sulphur were sucked up into a pipette together with some living Phacus triqueter, as previously described. This mixture of Phacus and sulphur was then fed on to the disk of a normally behaving Stentor in the fully extended condition, and the path and fate of each particle recorded. The results follow:

The particles are numbered in the order in which they reached the disk of the Stentor. The sulphur particles are denominated "s," the Phacus "p." Thus, 7s in the "rejected" column signifies that the seventh particle was sulphur, and that it was rejected. Where several numbers are bracketed, it signifies that these particles were fed simultaneously. In the column headed "loops" is shown the number of loops made by the particle. (See p. 11). The column headed "size" gives the size of the sulphur particles in units of the size of Phacus. Thus I, means same size as Phacus, .5 means one-half that size.

TABLE I

Experiment 1. Discrimination between Phacus and Sulphur

PARTICLES EATEN	PARTICLES REJECTED	LOOPS	SIZE	PARTICLES EATEN	PARTICLES REJECTED	LOOPS	SIZE
					15p		
1p					16p		
2p					17p		
3p				18p		2	
4p				19p		2	
5p				20p			
	6s		1	21p			
	7s		1	22p			
	8s	2	1	23p			
	9s	2	1	24p			
	10s	3	1		25s		1
11s		2	1		26s	3	·5
	12s		1		27s	3	·5
	13s		1		28s	3	·5
	14s		1		29s	3	·75

SUMMARY

Eaten, 12 Phacus and 1 grain of sulphur.
Rejected, 3 Phacus and 13 grains of sulphur.

In the above experiment there were eaten 12 Phacus and 1 grain of sulphur, while 13 grains of sulphur and 3 Phacus were rejected. It is evident therefore that in this case there is some sort of discrimination between Phacus and sulphur.

Experiment 2. Discrimination between Starch Grains and Phacus

In another experiment designed for the same purpose but in which iodine-stained potato starch grains and Phacus triqueter were used, even more sharply defined results were obtained. The technique was similar to that of the preceding experiment, except that two pipettes were used, one for starch and the other for Phacus. The starch was stained with iodine to facilitate observation. Previous to feeding, the starch was washed very thoroughly to remove all the superfluous iodine. The results are as shown in Table II.

This experiment shows conclusively that Stentor can and does discriminate between two kinds of particles differing as much as Phacus and starch grains do from each other. The possibility of coincidence is entirely ruled out of court in that the stream of particles was changed at least seven times from starch to Phacus and from Phacus to starch. In the whole experiment there are only four "mistakes" at the most, including the rejection of a swarmspore twice and of a Coscinodiscus. But later experiments will show that the rejection of the swarmspore and Coscinodiscus were probably not mistakes, so that there occurred in this test only one mistake, that of ingesting particle numbered 69—a starch grain. The size of the starch grains was variable, being from one-eighth to four times the size of a Phacus specimen. This shows that in this experiment, as in the preceding, size was not the determining factor in the selection. Another point worth noticing is that there are no loops in the paths of the particles swallowed, and that there are very few particles rejected without loops. The 45 loops of particle 32 are probably due to the fact that the Stentor was at that time lying on its side with pouch uppermost, thus making the removal of the particle more difficult than usual. When the animal turned over the particle was gotten rid of. The usual number of loops does not exceed 10 in any one case.

s = starch grains; p = Phacus; c = Coscinodiscus; sp = swarmspore. Where (—) occurs it signifies that the group in which it is found was broken; that is, some of the members of the group were eaten while the others were rejected. Thus, in group 36p, 37p, 38p, (—), which consisted of four particles fed simultaneously, the first three were eaten while the last one was rejected. Size of starch is given in terms of Phacus.

TABLE II

Experiment 2. Discrimination between Starch Grains and Phacus

EATEN	REJECTED	LOOPS	SIZE	EATEN	REJECTED	LOOPS	SIZE
1p				36p			
2p				37p			
3p				38p			
4p				(—)	39c		
5p				40p			
6p				41p			
7p					42s	2	4
8p					43s	3	.125
9p					44s	2	.5
10p					45s	2	.5
11p				46p			
12p				47p			
13p				48p			
	14s		1	49p			
	15s		1	50p			
	16s		1	51p			
	17s	3	1	52p			
	18s	6	1	53p			
	19s	3	1	54p			
20p				55p			
21p				56p			
22p				57p			
23p				58p			
24p				59p			
25p				60p			
26p				61p			
27p				62p			
	28s	8	1	63p			
	29s	8	1	64p			
	30s	3	.5	65p			
	31s	2	.5	66p			
	32s	45	1	67p			
	33sp	2			68s	3	1
34p				69s			.5
35p					70s	3	1
30 minute intermission					71s	3	1

SUMMARY

Eaten, 50 Phacus, and 1 starch grain.

Rejected: 18 starch grains, 1 Coscinodiscus, and 1 swarmspore.

To determine what would be the effect of feeding in a mixed stream three kinds of particles, two that were not food and one that was food, natural starch grains, powdered glass, and Phacus triqueter were sucked up into different capillary pipettes and fed to a normal Stentor with the following results.

Experiment 3. Discrimination between Starch Glass, and Phacus

p = Phacus; s = starch grains; g = particles of glass. The particles of starch and glass were selected of a size about equal to that of Phacus.

TABLE III

Experiment 3. Discrimination between Starch, Glass and Phacus

EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS
	1s			18s	38	35p		
	2s			19s	38		36p	11
	3s			20s	38	37p		
	4s		21p			38p		
	5s		22p			39p		
	6s		23p			40p		
7s			24p			41p		
8p			25p				42g	
9p				26p	3		43g	
10p				27p	8		44g	
11p				28p	2		45g	
12p				29p	4		46g	
13p				30p	3		47g	
14p				31p	3		48g	
	15s		32p				49g	
	16s		33p		4		50g	
	17s		34p		2		51g	
							52g	

SUMMARY

Eaten, 21 Phacus and 1 starch grain.
Rejected, 12 starch grains, 11 particles of glass, and 7 Phacus.

At this point the Stentor contracted and swam away. The discrimination in this experiment is of about the same degree of accuracy as in the two preceding experiments. The specimens of Phacus which were rejected may not represent a mistake in discrimination at all, but may be due to a condition of partial satiety,

which will be taken up a little later. If this is the case the Stentor discriminated very well indeed what was Phacus and what was glass or starch. An interesting feature of this experiment is the number of loops that are recorded for the Phacus which were rejected and the absence of loops in the paths of the particles of glass.

So far, these experiments show that Stentor can select Phacus from a stream of mixed particles in which there are one or two kinds of indigestible particles mixed with Phacus. There are two possibilities as to the way this selection is accomplished. First it may be that Stentor ingests from a mixed stream only one kind of food particles (such as Phacus triqueter), and rejects all other kinds of food and indigestible substances. The other possibility is that Stentor ingests all sorts of food particles and rejects all sorts of particles that are not food. To determine which of these alternatives is the one which actually obtains, the following experiment was performed in which two kinds of food particles, Phacus triqueter and Euglena viridis, were fed in a mixed stream with two kinds of indigestible substances, powdered sulphur and powdered glass. The following are the results:

Experiment 4. Selection of Phacus and Euglena from Sulphur and Glass

The Stentor upon which this experiment was tried was the same one which had submitted to the second experiment, on the previous day (p. 90). It still contained considerable amounts of partly digested food which probably represented the Phacus that were eaten the day previous. The starch grain which was eaten in Experiment 2 was not to be seen, and it is probable that it was voided some hours after it was ingested. That it was digested is made highly improbable in view of the work of Meissner ('88) who found that nearly all of the potato starch which the Stentors ate was not digested, and that some of the starch which remained in Stentors for over 48 hours was practically in the same condition as when fed. There is therefore no good ground for supposing that any part of the behavior of the Stentor in this experiment was due to the fact that a starch grain was ingested on the

previous day. As later experiments will demonstrate, however, the ingestion on the day before of the 50 Phacus has probably influenced the behavior of Stentor, and it is pretty certain that the condition of partial satiety which is exhibited in the beginning

p = Phacus; e = Euglena; s = sulphur; g = glass; t = encysted Trachelomonas volvocina.

TABLE IV

Experiment 4. Selection of Phacus and Euglenæ from Sulphur and Glass

EATEN	REJECTED	LOOPS	SIZE	EATEN	REJECTED	LOOPS	SIZE
	1p	5			21g	3	1
	2p	3			22g	3	2
	3p	2			23g	2	.5
4p					24g		1
5p					25g		1
	6p				26s		.25
7p					27s	2	.25
8p					28s	2	1
9p					29s	3	1
10p					30s	5	.5
11p					31s	5	.5
12p				32e			
13p				33e			
(–)	14p			34e			
(–)	15p				35t		
(–)	16p			36e			
(–)	17p			37p			
18p				38p			
19p				39p			
	20g	3	1	40p			

SUMMARY

Eaten, 15 Phacus and 4 Euglenæ.

Rejected, 8 Phacus, 6 particles of sulphur, 6 particles of glass, and 1 Trachelomonas.

of Experiment 4 was due to this cause. I think it will be pretty clearly shown that the rejection of the first three Phacus in Experiment 4 was not a "mistake" on the Stentor's part but probably represents a transition from a condition of partial satiety to one of hunger, brought about by the stimuli of the Phacus upon the pouch and funnel. The Phacus numbered 14, 15, 16, 17—the last four of a group of nine—were rejected probably because of the

inconvenience or impossibility of swallowing as many as nine Phacus at once. In very hungry Stentors six are sometimes swallowed at one gulp, and once a Stentor was observed to swallow seven, but in groups of more than seven some were always rejected.

So then in this experiment there are probably no mistakes in discrimination whatever unless the rejection of a Trachelomonas volvocina is considered a mistake. But the Trachelomonas was in the resting stage and inactive, as were also the swarmspore and the Coscinodiscus in Experiment 2. There is a possibility therefore that the inactive particles are rejected and that only moving, active particles (organisms), are ingested. To determine whether selection is made upon this basis, some Euglena viridis were taken from a culture and killed in various ways, by heat, alcohol, acetic acid, etc. They were then thoroughly washed and sucked up into capillary pipettes, those killed by heat in one pipette, those killed by alcohol in another, and so on. Another pipette was then filled with normal living Euglenæ. Normal Stentors were then isolated and fed with these Euglenæ in various conditions in a mixed stream, precisely as was done with the various substances in the preceding experiments. There was no discrimination observed between any of the differently prepared Euglenæ. The dead Euglenæ were eaten with the same readiness as were the living. Some Stentors rejected about equal numbers of each, while others rejected all of both kinds. Similar experiments were tried using Phacus and Trachelomonas as food, with the same results. There was no selection between the living and the dead organisms.

Another possible way of explaining how the rejection of the Trachelomonas, Coscinodiscus, and the swarmspore was made, is that Stentor may be able to distinguish the different kinds of food particles from each other, and that certain kinds of food may be eaten with more readiness than others. This kind of selection may, perhaps, take place under all conditions, or only under certain conditions. It may depend on the relative number of the different kinds of particles, or the order in which they come. To determine this matter a number of experiments were designed.

The first experiment was designed to show whether selection was exhibited when Phacus triqueter and Phacus longicaudus were fed in mixed order, Phacus triqueter being much more numerous than Phacus longicaudus.

Experiment 5. Discrimination between Phacus triqueter and P. longicaudus

(See Table V)

Out of the five Phacus longicaudus which were fed with the 176 Phacus triqueter, four were rejected and one was ingested. The four rejected ones were the last members of the respective groups in which they were fed, and the one which was ingested cane first in the group of two—*166l*, 167. It was not positively ascertained in any of my experiments whether discrimination is nicer among the last members of a group than among the first, but the evidence seems to point that way. But even if this should turn out not to be true, it seems clear that actual discrimination between Phacus triqueter and P. longicaudus took place in this experiment.

Experiment 6. Discrimination between Different Species of Organisms

In another experiment designed to further show selection of one or more kinds of food particles from as many as six different species of organisms, the following results were obtained. There were fed in mixed order, Euglena viridis, Euglena deses, Phacus triqueter, Phacus longicaudus, Trachelomonas hispida, and Trachelomonas volvocina. All these organisms were fed from a single pipette on to the disk of a Stentor as in the preceding experiment. For results see Table VI, p. 99.

Experiment 7. Discrimination between Different Species of Organisms

Immediately following the above experiment I fed another Stentor with the same sorts of flagellates (but omitting the Euglena deses which is difficult to handle in a capillary pipette owing to its habit of sticking to the walls) with the results shown in Table VI, p. 100. Loops are not recorded.

The numbers followed by *l* are Phacus longicaudus; all the other numbers represent Phacus triqueter.

TABLE V

Experiment 5. Discrimination between Phacus triqueter and Phacus longicaudus

EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS
1			45			89		
2			46			90		
3			(–)	47	7	91		
4			(–)	48	4	92		
5			49			93		
6			50			94		
7			51			95		
8			52				96	5
9			53			97		
10			54				98	4
11			55				99	3
12			(–)	56	6		100	3
13			57				101	
14			58			102		
15			(–)	59	21	103		
16				60	3	104		
17			61			105		
18			62			106		
19			63			107		
20			64			108		
21			65			109		
22			66			110		
23			67			111		
24			68			112		
25			69			(–)	*113l*	2
26			70				114	6
27			71				115	6
28			(–)	72	4		116	5
29			(–)	73	3		117	4
30				74	3		118	8
31				75	2		*119l*	3
32			76			120		
33			77			121		
34			78			122		
35			79			123		
36			80			124		
37				81	2	125		
38			82				126	4
39			(–)	83	3	127		
40				84	4		128	11
(–)	41	4		85	2	129		
42				86	5	130		
43				87	3	131		
	44	3		88	3	(–)	132	1

TABLE V—continued

EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS
133			149			*166l*		
(–)	134	3	(–)	150	6	167		
	135	1	(–)	151	4	168		
136			152			(–)	169	
137						170		
138				153	4	171		
139			155	154	4	(–)	172	4
140			156			(–)	173	4
141				157	1	(–)	174	1
142				158	1		175	2
143				159	8		176	2
144			160			177	*179l*	1
145			161			178		
146			162			(–)	*179l*	1
147			163				180	6
148			164					
			(–)	*165l*	1			

At first sight neither Experiment 6 or 7 seems to show that Stentor eats some kinds of food with more readiness than other kinds, for some individuals of each of the various kinds of flagellates were eaten while some of every kind were rejected. But upon closer examination it is found that the latter parts of the experiments are different from the earlier in several respects. A larger proportion are rejected at the end of each of the experiments than at the beginning. Of the organisms eaten the variety is much more extensive at the beginning than in the latter part of the experiments. All the organisms that were fed in the beginning of both experiments were ingested, but at the close only Euglena viridis and Trachelomonas volvocina were ingested, all the other kinds being rejected. Only a single Trachelomonas hispida was ingested. It is clear that there occurred a change in the physiologic state of Stentor as each of the experiments progressed. In the sixth experiment, of the six different kinds of flagellates that were fed, Euglena viridis and Trachelomonas volvocina were eaten with the greatest readiness. After the twenty-second organism had been fed, ten Euglenæ viridis were ingested and five were rejected. The fact that not all the Euglenæ were

ev = Euglena viridis; ed = Euglena deses; pt = Phacus triquetur; pl = Phacus longicaudus; th = Trachelomonas hispida; tv = Trachelomonas volvocina.

TABLE VI

Experiment 6. Discrimination between Different Species of Organisms

EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS
1pt			30ev				59pl	
2ed			(–)	31ev			60ed	
3ev				32pl		61ed	62pl	
4ev				33ev				
5ev				34pl			63pl	
6ev				35pl			64ch*	5
7ev				36pl			65tv	2
8pl				37pl			66tv	2
9ev				38pl			67pl	
10ev				39pl			68pl	4
11pl				40pl	5			
12pl			41ev			Three minute intermission. Pipette was refilled with food organisms.		
13pl			42tv					
14pl				43th				
15pl				44ev				
	16ev			45th			69ev	
	17pl			46th			70ev	
	18pl	2		47ev	4		71ev	
19ev				48pl			72ed	
20pt			49tv				73ev	
21pl			50ev				74ev	
	22pl	2	51ev				75ev	
23ev				52pl			76ev	
24ev				53th			77ev	
25ev			54ev				78ev	
(–)	26ev		55ev				79ev	
	27pl			56th			80ev	
	28pl		57ev				81ev	
	29pl			58th				

*Food particle 64 was a Coleps hirtus.

ingested does not prove that there was no selection going on in this part of the experiment, for the Phacus triqueter and the Trachelomonas hispida are without exception rejected. The explanation of this apparently capricious selection of some Euglenæ and the rejection of the rest is probably to be sought in the changed

condition of the Stentor brought about by the particles which were just previously ingested. That is, the Stentor was probably in a condition of partial satiety, where less and less food is taken even if it should meet all the requirements of a perfect food under conditions of hunger. The actual proofs of such conditions of partial and complete satiety will be taken up later on, but it may be pointed out here that in the sixth experiment the Stentor apparently grew less and less hungry as the number of ingested particles increased.

Particles designated as in Experiment 6

TABLE VII

Experiment 7. Discrimination between Different Species of Organisms

EATEN	REJECTED	EATEN	REJECTED	EATEN	REJECTED	EATEN	REJECTED
1pl		15pl		30ev		45tv	
2pl		16pl			31pl	46tv	
3pl		(–)	17pl		32pl		47th
4pt		(–)	18pl	33ev		48tv	
5pl		19pl		34pl			49pl
6pl		20pl		35ev		50tv	
7pl		21ev		36pl			51pt
8th		22pl		37ev			52pl
9ev		23ev		38ev		53tv	
10pt		(–)	24pl		39tv		54ev
11pl		25ev			40th		55th
	12pl	26ev			41pl		56th
13pl		27ev			42pl	57tv	
14pl		28pt			43pl		58ev
		29pl		44tv			59ev

The proportion of Euglena viridis and Trachelomonas volvocina was not the same in the two experiments, so no conclusions may be drawn with regard to which of these two kinds of organisms is eaten with the greater readiness. But it is of course clear that Euglena viridis and Trachelomonas volvocina are "preferred" by the Stentor to Phacus triqueter, P. longicaudus, and Trachelomonas hispida; which is only another way of saying that Stentor expresses a choice in the food which it eats.

Experiment 8. Discrimination between Organisms of Similar Size and Shape

As a final test in food discrimination in which the path and fate of each particle was recorded the following experiment was designed and performed. Two kinds of organisms were used, one being Trachelomonas volvocina and the other a species of Euglena which upon very slight disturbance contracted into a spherical mass of almost exactly the same size as that of a Trachelomonas. The organisms were fed from a capillary pipette in a mixed stream, the number of Euglenæ and Trachelomonas being as nearly equal as possible. The loops were not recorded. The results follow:

e = Euglena; t = Trachelomonas; th = Trachelomonas hispida

TABLE VIII

Experiment 8. Discrimination between Organisms of Similar Size and Shape

EATEN	REJECTED	EATEN	REJECTED	EATEN	REJECTED	EATEN	REJECTED
1e			16t		32e		47e
2e			17t		33t		48e
3e		18t			34t	49e	
	4e		19t		35e		50t
5t			20t		36e		51t
6t			21t	37e			52t
7e		22e			38t		53t
8e		(–)	23e		39t		54e
9c			24t	40e			55t
	10th		25t		41t		56t
	11e		26e		42e		57e
	12e		27e		43t	58e	
	13t	28e		44e			59t
	14t	29e			45t	60e	
	15t	30e			46t		61t
		31e					62e

Of the 62 organisms 32 were Euglenæ, 29 Tracheolmonas volvocina, and 1 T. hispida. Seventeen Euglenæ were eaten and 15 rejected; and of the Trachelomonas 3 were eaten and 27 rejected. That the Euglenæ were eaten with more readiness than the Trachelomonas is evident from an inspectoin of these figures, which

show that 56 per cent of the Euglenæ were eaten and only 10 per cent of the Trachelomonas. But a better and truer idea of the accuracy of selection is obtained by a study of the results as set forth in the table. As in the sixth and the seventh experiments, the beginning of this experiment differs from the latter part in that the variety of ingested particles is greater in the beginning, and also in that the proportion of food ingested as compared with the amount fed, is greater in the beginning. There seems also to be a marked change in the basis upon which discrimination is effected; it evidently became more restrictive towards the end.

What is the cause of the change in the degree of restrictiveness in selection in these experiments? Is the change due to differences in the food or to an alteration in the Stentor itself?

Evidently different food particles possess different strengths or powers of giving the stimulus that causes Stentor to ingest them. The stimulus from Phacus triqueter is stronger than that from Trachelomonas hispida; and that from Euglena viridis is still stronger than that from Phacus triqueter. This is shown by the fact that at certain times (when nearly replete), Stentor takes only Euglenæ (or Phacus if Euglenæ are not present), from a mixed stream of organisms. There probably are slight differences between the strengths of the stimuli from different individuals of the same species, but these differences are evidently less than those between different species, for when nearly satiated, Stentor takes only the particles of one species.

Yet we find in the experiments described above that specimens of the same organism (as Euglena) are at first eaten without any exception, while later less than half of them are eaten. The change in the proportions eaten as the experiment progresses are well seen in the following tabulation of the results of the eighth experiment. The experiment is divided into successive groups of about ten particles to each group.

TABLE VIII*a*

Tabulation of Results of Experiment 8

GROUPS	EUGLENAE EATEN		EUGLENAE REJECTED		TRACHELOMONAS EATEN		TRACHELOMONAS REJECTED	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
1–10	6	86	1	14	2	$66\frac{2}{3}$	1	$33\frac{1}{3}$
11–21	0	0	2	100	1	11	8	89
22–30	4	57	3	43	0	0	2	100
31–40	3	50	3	50	0	0	4	100
41–50	2	40	3	60	0	0	5	100
51–62	2	40	3	60	0	0	7	100

As this table shows, the percentage of Euglena eaten decreases steadily throughout the experiment, from 86 per cent to 40 per cent; while that for Trachelomonas decreases from $66\frac{2}{3}$ per cent to 0. The percentage of Euglena rejected increases from 14 per cent to 60 per cent; of Trachelomonas from 33 per cent to 100 per cent. Similar changes were found in all the experiments of like character with Stentor, including a number which I do not publish in detail. It is evident that this regular change cannot be due to chance variations in the food, but must be due to an alteration in the physiologic state of Stentor itself. In some way the Stentor changes as it takes food, so that stronger and stronger stimuli are required to set off the ingesting mechanism. Such a change is of course parallel with what we observe in higher organisms.

We find therefore that we have in this unicellular organism physiologic conditions corresponding to what we call hunger and satiety in higher forms. Differences in behavior due to hunger and satiety have not heretofore been demonstrated for those protozoa that secure their food by means of an alimentary vortex. The experiments we have just described, primarily designed to test the power of selection, indicate strongly the existence of such differences. Let us now turn to experiments that were planned to test this matter. We wish to determine what differences exist between hungry and satiated Stentors, and whether there are intermediate conditions, manifesting themselves in differences in behavior.

Experiment 9. Effect of Hunger on Behavior in Feeding

Eight Stentors with some of their own culture solution were placed into a small preparation dish. After they had attached themselves I selected a large individual and fed it with Phacus triqueter with the result shown in Table IX.

Experiment 10. *Effect of Satiety on Behavior in Feeding*

At the close of the foregoing experiment the dish of Stentors was set aside until next day when it was found that the Stentor which was fed in this experiment contained a considerable number of small brownish bodies. These upon close examination were found to be Phacus triqueter in a stage of partial digestion. The protoplasm was extracted from the Phacus and the chlorophyll was changed in color. Here was then a good opportunity to see whether the ingestion of Phacus on the previous day had any effect upon the ingestion of food particles today. A mixture of Euglena viridis and Phacus triqueter was fed from a capillary pipette on to the disk of Stentor with the results shown in Table X.

Perhaps the most notable difference between these two experiments is in the number of food particles ingested. In the ninth experiment 73 out of 118 particles were eaten, the majority being ingested in the first half of the experiment, while in the tenth only 10 were eaten out of 75, and these ten were distributed comparatively evenly. A large number of experiments performed upon well-fed Stentors showed substantially the same results as Experiment 10. In no case where the membranellæ and pouch cilia beat normally, i e., where their movement was not reversed, did it happen that every single particle was rejected. Some few were always ingested if the experiment was sufficiently extensive. The lowest ratio of particles eaten to those fed was 1 to 12. When the particles were fed very rapidly the ratio was very much increased. But if the particles are fed at the rate of about 100 an hour, results like those of Experiment 10 will be obtained. Stentors from artificial cultures in which food is very plentiful, and in which the Stentors thrive and reproduce very rapidly, show substantially the same results as are shown in Experiment 10.

TABLE IX

Experiment 9. Effect of Hunger on Behavior in Feeding

EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS
1				47	1		87	2
2				48	2		88	4
3				49	3	89		
4			Stentor contracted; hit with the			(-)	90	2
5			pipette.			(-)	91	3
6						92		
7				50	2		93	1
8				51	2		94	1
9				52	2		95	1
10		3		53	1		96	1
11				54	1		97	1
12				55	1	98		
13				56	5	99		
14			57			100		
15			58			(-)	101	1
16			59			(-)	102	2
17		6	60				103	1
18		4	61				104	1
19			62				105	1
20			63				106	1
21			64				107	6
22			(-)	65	2	108		
23			(-)	66	2		109	7
24			67					
25			68			At this point a Stylonychia was		
26		4	69			fed twice to the Stentor but was		
(-)	27	7	70			rejected each time. On being		
28			71			fed the 3d time the Stentor held		
29			72			the S. in the pouch for an instant		
30			73			and then contracted, setting the		
(-)	31	5	74			S. free. The S. was fed again		
(-)	32	5	75		1	to the Stentor and was eaten.		
33			(-)	76	4	The Stentor then contracted for		
34			77			a few minutes.		
35			78					
36			79				110	1
37			(-)	80	2		111	1
38			81		6		112	1
(-)	39	3	82				113	1
40			83		2		114	1
(-)	41	1				115		
42			Contracted; hit with the pipette.			(-)	116	2
43						117		
(-)	44	1	84			(-)	118	1
(-)	45	3	85					
46			(-)	86	2			

e = Euglena viridis; p = Phacus triqueter

TABLE X

Experiment 10. Effect of Satiety on Behavior in Feeding

EATEN	REJECTED	LOOPS	EATEN	REJECTED	LOOPS
	1e	1		44e	1
	2p	1		45e	1
	3p	1		46e	1
	4e	1		47e	1
	5e	1		48e	2
	6e	1	48 fed again	(49e)	3
	7e	1	50e		
	8e	1		51p	1
	9e	1		52p	1
	10e	1		53p	1
	11p	4		54p	1
	12e	2		55p	1
13e				56p	1
14e				57p	1
	15e	1		58p	1
16e				59e	3
(-)	17e.	1		Bit of Debris	1
	18e.	1	Fed again	(Bit of Debris)	1
18 fed again	(19e)	1		60p	1
18 fed again	(20e)	1		61p	1
18 fed again	(21e)	1		62p	1
18 fed again	(22e)	1		63p	1
18 fed again	(23e)	1		Bit of Debris	1
18 fed again	(24e)	1		64p	1
18 fed again	(25e)	1		65p	1
18 fed again	(26e)	3		66p	1
18 fed again	(27e)	2		67p	2
18 fed again	(28e)	1		68p	3
18 fed again	(29e)	1			
	30e	1	At this point the Stentor contracted. When again expanded, Stentor reversed the cilia when the stream from the pipette reached the disk. After about a minute the cilia beat normally.		
31p					
(-)	32e	1			
33e					
(-)	34e	1			
	35e	1		69e	2
	36e	2		70e	1
	37e	1	71e		
	38e	1	72e		
	39e	1	(-)	73e	3
	40e	2	(-)	74e	1
	41e	1		75e	1
42e					
43e					

Perhaps the most significant difference of all between the ninth and the tenth experiment is with regard to the occurrence of loops in the paths of the organisms which were fed. These two experiments are typical in this respect of nearly all the experiments which form the basis for this paper.

We find, first, that nearly all the loops occur in the paths of the rejected particles. As it happens in these two experiments, no particle is rejected without at least one loop in its path. Second, when loops occur in the paths of ingested particles they are generally found only in those experiments where a comparatively large proportion of the particles are ingested, or in other words, when the Stentor is hungry or only in the first stages of satiety. Third, in extensive experiments like the two preceding, more loops occur in the first half of the experiments than in the last half, both in the ingested and in the rejected particles. Fourth, very few loops occur in feeding hungry Stentors. Fifth, very few loops occur in a satiated Stentor. Sixth, the maximum number of loops is found when the Stentor is in the first stages of satiety. Seventh, in a stream of mixed particles including food and bits of glass or sand, the glass or sand is generally rejected with fewer loops than the food particles. A conspicuous case of this is seen in the third experiment.

What is the real significance of these loops? Are loops the result of fatigue of the ingesting or of the rejecting mechanism, or are they correlated with a certain physiologic state of Stentor in such a way as to be a factor in the selection of food? It appears clear that the loops are not due to fatigue of the ingesting or rejecting mechanisms, nor of the apparatus for receiving the stimuli. Fewer loops are made when the Stentor is satiated than when only partial satiety sets in; this would not be the case if the occurrence of loops were due to fatigue, as will presently appear. Further, when excessively small particles are fed, such as the Euglenæ in the eighth experiment, as many as 11,000 may be eaten and several times that number rejected, all within two or three hours, so that fatigue could hardly occur from handling a few hundred as in Experiments 9 and 10. Again we have seen that discrimination is more precise as the Stentor becomes satiated. This shows that

the apparatus for receiving stimuli has not become effectively fatigued.

Thus it appears clear that the occurrence of loops is not due to fatigue; we must look for the explanation in some other change in the physiologic state of the animal. As noted above, the loops appear as the animal approaches satiety, so that there can be little doubt but that the change in the degree of hunger is what brings on the loops. The loops, as we have seen, almost always occur in the paths of particles that are finally rejected. It appears that the rejecting mechanism is not set in operation by the first slight stimulus from an objectionable particle, but the stimulus seems to be summated with every successive loop that is made, until it is finally strong enough to cause rejection. In the first stages when the animal is hungry the ingesting mechanism is more readily set off than that for rejection; near repletion, the rejection apparatus is more readily set off; and as repletion advances the rejecting apparatus is continually more and more readily set off, requiring therefore fewer loops. The apparatus for receiving stimuli seems therefore to be in a state of continual change, so that stimuli which readily set off the ingesting reaction when the animal is hungry have but a slight effect when the Stentor is nearly replete, and finally have no effect at all, or set off only the rejecting reaction.

A difference is seen in the fact that when a particle is accepted, this is done (as a rule) at once, without loops, while rejection is done only after some delay, with the occurrence of loops. Only when the Stentor is fully satiated does rejection occur instantaneously. In the state of incomplete satiety, rejection is a slow and uncertain process, as if the stimulus for rejection had first to gather strength before rejection could occur.

Thus the number of loops which occur before rejection depends on the degree of hunger. There is doubtless a similar though reverse effect of hunger on the ingesting apparatus; it seems certain that a stronger stimulus is required to set off the ingesting reaction when the Stentor is partially satiated than when very hungry. But the evidence is not so clear as for the rejecting apparatus owing to the fact that loops rarely occur in the path of

a particle that is to be ingested. The nature of the assumed rejecting and ingesting mechanism and the way selection is brought about, will be discussed later on.

We have thus far dealt mainly with what may be called stages of moderate hunger and moderate satiety. There still remain the states of utter hunger and of surfeit to be described.

The condition of extreme hunger may be produced by putting a number of Stentors with as little of their culture solution as possible into clear tap-water and keeping the water as free from bacteria as possible. There is no evident difference in behavior between Stentors in the condition of extreme hunger and those moderately hungry.

The condition of utter satiety is more interesting. The behavior of a Stentor in such a state is very different from the normal behavior. The following experiment fully illustrates this. Several hundred small paramecia were placed with a few Stentors in a watch glass for two days, so that the Stentors could feed upon them. The largest of the several Stentors was then selected for feeding with Trachelomonas hispida. The Stentor was filled with a number of globular masses of slightly brownish transparent material which probably represented as many broken down paramecia. The Trachelomonas were fed from a capillary pipette as usual. Following are the results.

Experiment 11. The Effect of Utter Satiety on Stentor

In this experiment the figures, 1 to 24, signify as many Trachelomonas hispida which were fed to Stentor. As in preceding experiments, they are numbered in the order in which they were fed. Stage "pipette presented," means that the end of the capillary pipette which contained Trachelomonas hispida was brought within about half a millimeter from the animal's disk, and that a very slow stream of water was then caused to flow against the disk. All the other stages of behavior are self-explanatory.

Pipette presented
1 Trachelomonas hispida rejected with 1 loop
Contraction
All cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Contraction for 4 minutes
Cilia normally active
Pipette presented
Contraction
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Pippette removed
Cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Contraction
Cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Contraction
Cilia normally active

Pipette presented
Slowing of cilia
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Pipette removed
Contraction
Cilia normally active
Pipette presented
Slowing of cilia
(Pipette continued)
Bending away
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Bending away
Pipette removed
Cilia normally active
Pipette presented
Slowing of cilia
Bending away
Pipette removed
Cilia normally active
Put a Trachelomonas on the disk near the pouch with a very thin glass rod.
2 rejected, with 5 loops.
Pipette presented
Slowing of cilia
Pipette removed
Cilia normally active
Trachelomonas presented with glass rod.
3 rejected with 7 loops
Trachelomonas presented with glass rod
4 rejected with 4 loops
Pipette presented

Slowing of cilia
Pipette removed
Cilia normally active
Trachelomonas presented with glass rod
5 rejected with 4 loops
Pipette presented
Slowing of cilia
Pipette removed
Cilia normally active
Pipette presented Stream very slow
6 rejected with 3 loops
7 eaten
8 rejected with 4 loops
9 rejected with 8 loops
10 rejected with 4 loops
11 eaten
12 (Coscinodiscus) eaten
13 rejected with 8 loops
14 rejected with 4 loops
15 rejected with 8 loops
16 rejected with 5 loops
17 rejected with 3 loops
18 rejected with 3 loops
19 rejected with 9 loops
20 rejected with 3 loops
21 rejected with 4 loops
22 rejected with 5 loops
23 rejected with 7 loops
24 rejected with 2 loops

This Stentor was at no time as fully extended as the average Stentor is when not containing much food, nor was the ciliary action quite so strong and vigorous as when normally hungry. This experiment is a good example of a remarkable change in the physiologic state of Stentor in that the Stentor was slowly brought from a condition of surfeit to one of only partial satiety. The change was a gradual one inasmuch as five Trachelomonas impinged upon the disk before the change was complete. A single Trachelomonas caused apparently no visible change in behavior. There seems to have been required the summated stimuli from five Trachelomonas before the state of surfeit could be changed to one of only partial satiety.

There was also a gradual decrease in irritability arising from the stream of water from the pipette. At first this caused contraction. A little later only a slowing of cilia occurred—the initial stage of contraction. Still later the Stentor bent away from the source of the stimulus, and finally the stream from the pipette no longer caused any visible reaction.

This decrease in irritability is parallel with the decrease in satiety but is probably not due to the same cause. The cause of the decrease was probably the frequent repetition of the stimulus, the faint stream of water from the pipette, for a marked decrease in irritability resulted before the glass rod was used. But the

change from a condition of surfeit to one of only partial satiety was caused by the impinging of five Trachelomonas on the Stentor's disk.

This decrease in the condition of satiety does not represent a similar rate of decrease of food in the Stentor's body. We see therefore that a particular state of hunger in a Stentor does not directly nor necessarily accurately represent the amount of food in the Stentor at the given moment. What the state of hunger actually represents is the condition of the organ for receiving stimuli from external food particles. This is influenced: (1) by the past history of the amount and kind of stimulation from external particles; (2) by the amount of food in the Stentor's body.

Other peculiarities of behavior attendant upon the condition of satiety are the following:

1 Extension is always sub-maximal. Instead of being extended as fully as possible with the disk spread so as to present the greatest area to the base of the vortex set up by the membranellæ, the Stentor is only partially extended and the disk is smaller. The animal does not extend perpendicularly upward or horizontally from its base of attachment, but generally hangs downward, or frequently lies upon some debris, etc., if possible.

2 The aboral side is more strongly convex when replete than when hungry. This posture may be related in some way to the voiding of excrementa, though no evidence could be obtained to show that this is true.

3 There is a marked decrease in the activity of the membranellæ. This may have much to do with the degree of extension in Stentor. Strong action of the membranellæ tends to pull the disk away from the foot, and therefore full extension may be partly due to the strong beat of the membranellæ. If the membranellæ beat only in a weak manner there is no such pull upon the Stentor, and as a result it lies prone or hangs downward from its point of attachment. This is made still more probable by the fact that hungry, free-swimming Stentors are seldom as fully extended as attached ones.

4 Satiated Stentors are very irritable to stimuli affecting the swallowing or rejecting mechanisms. In hungry Stentors one

can poke around the pouch a good deal before a Stentor contracts, but in a satiated specimen the faintest touch generally causes contraction. But mechanical stimuli on the sides of the body do not seem to cause contractions more readily in replete than in hungry Stentors.

5 If the stimuli are not too strong, contraction is often resolved into stages, and only the first of these may be passed through, instead of all of them as is the case when the Stentor is hungry. Thus contraction may be resolved into the following separate stages. (*a*) Cessation of action of the membranellæ. (*b*) Closure of the pouch. (*c*) Gradual rounding up of the anterior and posterior portions of the Stentor into an oblong mass. If the stimulus still continues, complete contraction follows; but this act changes the form and size of the already partially contracted Stentor very little. No such slow contraction takes place in a hungry Stentor where, if the mechanical stimulus is strong enough to cause cessation of action of the membranellæ, the entire process of contraction occurs instantaneously.

EXPERIMENTS WITH MIXTURES OF PARTICLES

In this part will be considered experiments in which the food particles were not fed and observed individually, but in which the Stentors were surrounded by mixtures of particles of various kinds. The purpose was to determine whether the animals can make a selection from among the different particles of such a mixture. After remaining for various periods of time in such mixtures, the Stentors were placed on a slide and compressed with a cover glass. It was then possible to estimate with some accuracy the relative amounts of the various kinds of particles that had been ingested. For such experiments we can use only particles that remain long in suspension, so as to maintain their relative distribution. Heavy particles cannot be employed.

It is obvious that this method gives less precise results than employed in the foregoing experiments. But by its use certain additional problems can be attacked. We have seen that Stentor discriminates between different sorts of particles fed in

succession; can it also make a selection from among particles that are intimately mixed? And why does it at times take particles that are not good for food, such as carmine?

In the first series of experiments the following procedure was adopted. In each of several small preparation dishes there was placed 10 to 20 cc. of filtered fluid from the Stentor culture, together with about 30 Stentors that contained no solid food. Into some of the dishes was introduced a mixture consisting half of carmine particles, half of an admixture of Chlamydomonas with some very small Euglenæ. In others, serving as control carmine alone was introduced, in amount equivalent to the total quantity of particles in the other dishes.

The contents of the dishes were thoroughly stirred, and they were then placed in a dark box to prevent the Chlamydomonas from collecting at the lighted side of the dish. After half an hour the Stentors were examined under the microscope. The average content for each Stentor was about 1500 Chlamydomonas, about 85 Euglenæ, and carmine of the bulk of about 10 Euglenæ. Several Stentors had ingested about the same amount of food, but no carmine whatever.

About half the Stentors were left surrounded with these substances for 24 hours. These then contained a much greater amount of Euglenæ and Chlamydomonas than before, but in about the same proportions. But the carmine content was practically nil. This is probably explained by the fact, brought out in the first series of experiments, that discrimination becomes more perfect as hunger becomes less. Having become in the later hours nearly satisfied, the Stentors discriminated more accurately against the carmine, and meanwhile that which they had ingested in the first hours of the experiment had been egested in the natural course of events. The result was not due to the settling of the larger particles of carmine to the bottom, since when I added fresh carmine, none of it was ingested. It is also improbable that the Stentors had become "educated" to the fact that carmine is not food, as will be shown later.

In the control dishes where only carmine was present, the Stentors had ingested an amount of carmine equal to about two-thirds

of the quantity of substance taken in by the Stentors in the dishes containing both food and carmine. There was variation among different individuals in the amount taken, but none were entirely devoid of carmine.

A similar series of experiments in which india ink was substituted for carmine gave the same results, except that only about half as much india ink was ingested as carmine.

These experiments show that in an intimate mixture Stentor can discriminate and select with a high degree of accuracy such minute food particles as Chlamydomonas from among indigestible particles like carmine or india ink. In some cases the accuracy of selection is almost perfect. Further, it is seen that the larger amounts of carmine are eaten when no food is present, while little or none is ingested when food is present also.

In another experiment the Stentors came from a culture where food was more abundant. They were fed with mixtures containing one or more of the following: yeast, carmine, india ink, Euglenæ, Trachelomonas. Nine different combinations of these materials were made in separate dishes. Equal quantities of the different substances were employed in each experiment. At the end of an hour the Stentors of each dish were examined as to the materials ingested. The different combinations employed and the results obtained are given in the following series.

1 Mixture of yeast and carmine. Result: Stentors at the end of an hour contained about twice as much yeast as carmine. The total bulk ingested was equal to that of about 600 Euglenæ.

2 Yeast and india ink. Result: Less yeast ingested than in 1. Very little ink was eaten—about the bulk of three Euglenæ.

3 Euglenæ, Trachelomonas, and carmine. Result: Many Euglenæ and Trachelomonas ingested. Carmine to the bulk of about 15 Euglenæ.

4 Euglenæ, Trachelomonas, and ink. Result: Same quantity of Euglenæ and Trachelomonas a in 3. Ink to the bulk of 1 Euglena.

5 Yeast, Euglenæ, Trachelomonas, and carmine. Result: More Euglenæ and Trachelomonas then yeast. Carmine to the bulk of 9 Euglenæ.

6 Yeast, Euglenæ, Trachelomonas, and ink. Result: More Euglenæ and Trachelomonas than yeast. No ink.

7 Ink. Result: Ink to the bulk of 3 Euglenæ.

8 Carmine. Result: Carmine to the bulk of 20 Euglenæ.

10 Yeast, Euglenæ, and Trachelomonas. Result: More Euglenæ and Trachelomonas then yeast.

Only about half the Stentors of each dish were examined at the end of an hour. The others were allowed to remain in the dishes until next day. Examination then showed somewhat the same results as are described above. In dish 4 however, no ink was found in any of the Stentors, and in all the dishes in which ink and carmine were placed the amount of these substances ingested by the Stentors was less. The amount of yeast in the Stentors in dishes 6 and 5 was also considerably less than on the previous day. This decrease may have been due, in part at least, to the torulas sinking to the bottom, notwithstanding the frequent stirrings to which the dishes were subjected.

These experiments show that Stentor can discriminate between the torulas of yeast and the grains of ink or carmine, and that selection is more perfect for example, between yeast and ink than between yeast and carmine They show also that less ink and carmine are eaten when food is present than when not. But in the case of dish 1 the Stentors are found to contain more carmine when the yeast is also present than when only carmine is present as in dish 8. I am unable to explain why this is so in this particular case.

These two sets of experiments are probably sufficient to show that Stentor can discriminate as well when enormous numbers of particles touch the disk and pouch continuously for 24 hours, as when a small number are swept into the pouch in rather slow succession, and that very minute particles of different sorts can be sorted as accurately and rapidly as large particles. But all these experiments were performed when the different particles were mixed in about the same proportion. The question next came up: What will happen when the proportions are changed? Will Stentor select food particles from particles that are not food as accurately when the latter are greatly in excess? or in a mixture of different

kinds of food particles, will the ratio of ingesta vary directly with the proportion of the amounts of the different particles as they occur in the mixture? or does a variation in the different kinds of particles, in the matter of proportion, have no effect upon the amount of each kind ingested?

Trachelomonas volvocina, T. hispida, Phacus longicaudus, P. triqueter, Euglena viridis, and E. deses were placed in a small preparation dish with about 10 cc. of filtered Stentor culture solution. About 100 times as much carmine as food organisms was added and into this mixture several Stentors were placed, and the dish set in the dark for about an hour. Examination at the end of that time showed that about ten times as much food was eaten as carmine. This is practically the same result that was obtained in dish 3, described above, where the proportions of Euglena, Trachelomonas, and carmine were equal.

Another series of experiments bearing upon this point was performed with mixtures of a species of Euglena and a colorless flagellate, probably a species of Chilomonas. The Euglenæ were about 30μ long and about 12μ wide; Chilomonas, 18μ long and 8μ wide. These two organisms were mixed in various proportions. Stentors were then introduced and the dishes set in the dark for two hours. The contents of the dishes, together wi h the results obtained, follow:

1 Mixture of Euglenæ and Chilomonas in proportion of 4 : 1. Result: Ten times as many Euglenæ ingested as Chilomonas.

2 Chilomonas and Euglenæ in proportion of 4 : 1. Result: Four times as many Euglenæ ingested as Chilomonas. One typical Stentor contained 200 Euglenæ and 54 Chilomonas in 80 vacuoles.

3 Chilomonas and Euglenæ in same numbers. Result: About six times as many Euglenæ ingested as Chilomonas.

4 Many Chilomonas. Result: Stentors contained as many Chilomonas as Stentors in dish 6 contained Euglenæ.

5 Few Chilomonas. Result: Comparatively few Chilomonas eaten.

6 Many Euglenæ. Result: Many Euglenæ eaten,—from 5,000 to 11,000.

7 Few Euglenæ. Result: Comparatively few Euglenæ eaten.

Some peculiarities of behavior of the Stentors in the above series of experiments may be worth mentioning. About 60 per cent of the Stentors were swimming freely. In dishes 4 and 6 where the Chilomonas were dense, the Stentors swam continuously with the foot ahead. About one-third of all the free-swimming Stentors in dishes 2 and 4 (where the Chilomonas were dense) were in a state of almost maximum contraction. These Stentors contained very few Chilomonas. Nearly all the free-swimming Stentors in the other dishes were extended. Some of the attached Stentors in the dishes 2 and 4 were maximally contracted. These contained the smallest number of food organisms. Some of the other attached Stentors rolled masses of Chilomonas on the disk in the "push ball" fashion as described on page 86. The other attached Stentors in these dishes continually reversed their cilia. The attached Stentors in the Euglena dishes, 1, 3, 6 and 7, kept all the body cilia beating forward, whether all the Euglena were rejected or all eaten.

We have seen that Stentor takes less waste matter (carmine, etc.) when food is present, more when it is absent. This brings up another interesting question. In order that the animal shall reject substances not good for food, must it first have taken a certain amount of food? If so, how much food must be taken before discrimination begins? And in order to induce discrimination, is it necessary that food should be actually ingested, or is mere contact with food all that is required?

As to the quantity of food necessary to induce discrimination the experiments just described show that Stentor discriminates against carmine (or Chilomonas), about as completely when the proportion of Euglenæ present is very small (about 1 : 100) as when more Euglenæ are present. It is impracticable to work with smaller proportions of Euglenæ, since in such cases one cannot keep the various kinds of particles uniformly distributed.

To determine whether actual ingestion of food, or only contact with it, is necessary to induce discrimination, the following experiment was tried. The waste material, carmine, was mixed with rather large paramecia. The latter may serve as food for Stentor,

but they are rarely captured, though they frequently come in contact with the Stentors. Five dishes contained equal amounts of tap water and carmine particles; into some of these there was introduced also certain quantities of food particles, in others none. Fifty Stentors were placed in each of these dishes, and then were examined after 20 minutes to determine the relative amounts of carmine taken. The conditions and results are as follows.

1 Carmine alone. At the end of 20 minutes the Stentors contained carmine to the bulk of about 400 Euglenæ.

2 Several thousand paramecia plus carmine.

3 Several hundred paramecia plus carmine.

In dishes 2 and 3 none of the Stentors contained any paramecia. Their carmine content was about half that of the Stentors of dish 1, a bulk equivalent to that of about 200 Euglenæ.

4 Trachelomonas hispida, T. volvocina, and carmine. The Stentors contained a very few specimens of Trachelomonas. Carmine to the bulk of about 80 Euglenæ.

5 Euglena viridis and carmine. The Stentors had in 20 minutes taken each about 275 Euglenæ, and carmine equivalent in bulk to about 4 Euglenæ.

These results seem to indicate that the presence of the paramecia in dishes 2 and 3 decreased the amount of carmine taken up by the Stentors. The paramecia frequently touched the disk, and were often swept into the Stentors' pouches but always escaped, and it is probable that this touching of the discal or pouch cilia by the paramecia altered the physiologic condition of the Stentor in much the same way as if several paramecia had been eaten. It might be thought that the bacteria carried over with the paramecia produced the result described above, but this is probably not the case, since in many experiments of this nature carmine was put into a portion of filtered paramecia or Stentor culture solution which was full of bacteria, and yet the maximum quantity of carmine was taken up.

The evidence thus indicates that it is only necessary for food particles to touch the Stentor's disk or pouch in order that Stentor should then to some extent reject the waste particles.

A question related to the one we have just considered is the fol-

lowing. Does not Stentor react or behave differently to such indigestible particles as carmine or india ink after being in contact with them for a few minutes or half an hour? In other words, may not the Stentor under these conditions be "educated" into rejecting carmine after having for some time ingested this substance?

The paper by Metalnikow, referred to in the introduction, bears upon this very point. This author worked upon paramecium and found that if these organisms are fed with carmine for 15 days or so, they cease to take up this substance. "Füttert man Paramecien sehr lange Zeit hindurch nur mit einem Farbstoff, z. B. mit Karmin, so kann man häufig nachstehende interessante Erscheinung beobachten. Anfangs, während der ersten Tage des Fütterns, bilden sich in einem jeden Infusor 30-40 kleine, gaf ärbte, Karmin enthaltende Vacuolen. Hierauf nimmt die Zahl der Vacuolen immer mehr und mehr ab. Bereits nach wenigen Tagen enthält ein jedes Infusor nicht mehr wie 15-20 Vacuolen, während nach 10-15 Tagen nicht wenige Infusorien angetroffen werden, welche keine einzige gefärbte Vacuole mehr enthalten. Nach einem noch grösseren Zeitraum hören sämmtliche Infusorien auf Karmin zu verschlucken" (*l. z.*, p. 183, 184).

I attempted to verify Metalnikow's results upon Stentor and expected at the outset to find perhaps a greater capacity in Stentor to acquire such a "ganz neue Fähigkeit" than in Paramecium, because of the generally accepted notion that Stentor is more highly developed as far at least as its "action system" is concerned. It would be in line with the results obtained by my other experiments on Stentor. But I was disappointed, as the following experiments show.

In 5 large covered dishes were placed 60 watch glasses, each one being filled with about 5 cc. of fresh, weak, filtered hay infusion. In 6 of these glasses were placed Stentors; in 6 more were placed Stentors and paramecia; ratio, 1 :10; 6 others contained Stentors and Euglenæ, 1 :100; 6 others contained Stentors, paramecia, and india ink; 6 others, Stentors, paramecia, and carmine; 3 others Stentors and india ink; 3 others, Stentors and carmine; 6 others, paramecia; 3 others, paramecia and india ink; 3 others, paramecia and carmine; 6 others, paramecia and Euglena; 3 others, para-

mecia, Euglenæ, and carmine; 3 others, paramecia, Euglenæ, and india ink.

One hour after these experiments were set up all the glasses were examined. All the Stentors which were placed in ink or carmine mixtures, whether paramecia were present or not, contained ink or carmine but the amounts were variable. More carmine was found in the Stentors than ink. Likewise all the paramecia placed in ink or carmine mixtures contained vacuoles of these substances. The highest number of vacuoles was from 40 to 50. The lowest number observed was 4. More ink than carmine was found in paramecia.

All the glasses were examined once a day after they were set up. Three days after the experiment was begun, some paramecia were found with very few vacuoles of carmine. The smallest number was 4. These paramecia were then placed in fresh carmine, and in half an hour they contained 50 or more vacuoles each. The carmine and ink content of the Stentors had so far remained constant. Seven days after the experiment was set up, no decrease in the number of vacuoles of ink or carmine in either paramecia or Stentors was found. But one paramecium was found with no carmine vacuoles. This paramecium was then placed with fresh carmine and for over an hour no carmine was taken. It was then placed in fresh ink and in 30 minutes 6 vacuoles of ink were found to have been ingested. This result is probably due to the fact that the grains of india ink are smaller than the grains of carmine.

Ten days after the experiment was set up many paramecia in those glasses where the least ink and carmine happened to be, contained very few vacuoles of these substances, but upon adding some fresh carmine or ink these paramecia at once filled up on these substances as they had done in the beginning of the experiment. The paramecia in the glasses where the carmine or ink was dense contained about the same quantity of these substances as at the start. About the same may be said for the Stentors. In the glasses in which the ink or carmine was dense the Stentors were filled with these substances. In other glasses the Stentors contained very little ink or carmine; but upon refilling, these substances were again taken up as at the beginning of the experiment.

Fifteen days after the experiment was begun, Raphidium developed in the glasses and the Stentors died. About six of the dishes which contained paramecia and ink or carmine were however in good condition. These paramecia showed no decrease in the number of vacuoles of these substances. After two or three days these paramecia also died.

These results are quite at variance with those described by Metalnikow. I therefore contrived another experiment which eliminates certain possibilities of error to which the above experiment and those of Metalnikow were open. A clinostat was set up with a cylindrical jar revolving on the horizontal axis once in every 15 minutes. Seven small vials were then filled with Stentors and paramecia in 5 cc. of their own culture solution. In 3 of these vials was placed carmine; in the other 3 india ink; and the other was left for a control. The vials were corked and placed loosely in the jar revolved by the clinostat. As the clinostat revolved the jar, the vials rolled around, and as they did so their contents were shaken up thoroughly about three times every 15 minutes. In this way the contents of the vials were distributed uniformly for the thirty-three days throughout which the experiment was carried on. The Stentors, paramecia, and culture solution were placed in the vials in the same proportion in which they existed in nature. A little fresh carmine or ink was added every few days. The contents of the vials were examined every day, whenever possible, in this manner. The contents were poured out into a small preparation dish and placed under a binocular microscope. The condition of the Stentors and paramecia was then easily observable. In this way the water in the vials was also thoroughly aerated. About 30 Stentors and 500 paramecia were placed in each vial. The following are the tabulated results for the paramecia. The table shows the number of paramecia which contained no ink or carmine on the dates shown.

		OCTOBER									NOVEMBER												
DATES.....		22	23	24	26	27	28	29	30	31	4	5	6	7	9	10	11	12	13	16	18	20	23
Carmine vials	*a*	4	2	6	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	2	3	2	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	c	3	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ink vials.....	*d*	2	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0				
	e	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0							
	f	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Many paramecia in the act of dividing were observed to have from 5 to 15 vacuoles in them. Only one paramecium was found to contain no ink or carmine in dividing.

The paramecia of November 4 and 12 which had no carmine or ink in them were of abnormal, boomerang shape. The number of vacuoles in the paramecia in these vials was almost constant as long as the experiment was carried on—over 33 days. Attempts were made at counting vacuoles and the average high number was about 70. About 5 per cent of the paramecia were found to conta n only from 5 to 15 vacuoles. In a number of cases such individuals were isolated and fresh carmine or ink added, when they would invariably fill up again. Ink was generally eaten more readily than carmine. The paramecia acted normally as long as the experiment was continued, and there was reason to believe that the experiment could have been continued further if it had been deemed necessary.

As for the Stentors, throughout the entire run of the experiment, none contained neither ink nor carmine. It was impossible to count the vacuoles because of their irregular sizes and shapes. The ink and carmine content of the Stentors was fairly constant. No decrease in the amounts of these substances in the Stentors was found throughout the entire experiment.

It is clear from these experiments that if either Stentor or paramecium can be "educated" in such a way as to refuse to eat carmine, etc., after feeding carmine to these organisms for a month, it can only be done under very special conditions. Metalnikow does not describe his experiments in detail, but from what

I was able to gather from his paper with regard to the method of work our results disagree utterly.

What is the reason for the disagreement? Metalnikow says that after his paramecia had ceased to take up carmine he stirred up the solution and yet the paramecia refused to ingest any more carmine. In my work I also found this to be true, but I attributed this to the fact that the carmine is no longer mixed in the same way as it was originally, for the mucus excreted by the paramecia, and other colloidal matter in the water, cause the carmine particles to stick together, so that the paramecia no longer take this kind of carmine with readiness. Further Metalnikow states that if one takes some of these paramecia which have refused to eat carmine and places them in fresh carmine, none is taken up. I have also tried this many times, but in almost every case I found that the paramecia again took up fresh carmine. In some few cases I found paramecia which did not eat carmine under any circumstances, even if they were placed in fresh solutions, but close examination nearly always showed that these rare individuals either were deformed or had just previously divided, and so were unable structurally to ingest the grains of fresh carmine. It appears possible that Metalnikow unfortunately got hold of some of these individuals.

But there is another statement in his paper which is hard to reconcile with the one quoted above.

"Hält man die Infusorien bei mässig hoher Temperatur (d. h. bei 10-15° C.; die Versuche wurden während der kalten Jahreszeit angestellt), so tritt während mehrere Tage keine Theilung ein.

"Jeden Tag untersuchte ich ein solches ungeteiltes Infusor auf seine Fähigkeit hin Karmin zu verschlucken. Im Verlauf von zwei, dre (in seltenen Fällen auch 4) Tagen, während welcher Zeit es mir gelungen war die Infusorien von einer Theilung abzuhalten, konnte ich nicht bemerken, dass ihr Verhalten zum Karmin irgend welche Abänderungen erfahren hätte. Jeden Tag weigerten sich die Infusorien hartnäckig Karmin zu fressen. Kaum aber hätte sich ein Infusor geteilt, so änderte sich sein Verhalten plötzlich und es begann von neuem Karmin zu fressen. In denjenigen Fällen wo ich das Infusor in den Thermostat oder an

einen warmen Ort (von über 20° C.) vebrachte, trat eine Theilung meistens sehr bald ein und die Tochterinfusorien begannen sofort Karmin körnchen zu verschlucken."

Metalnikow thus finds that although paramecia learn in some way to refuse carmine after being fed with it for some time, yet as soon as they divide, the daughter paramecia at once take up carmine as before. But he also says that after paramecia had been left in carmine for 15 days or longer, most of them contained no carmine whatever. Whether there were only very few paramecia dividing in this latter case, or whether the paramecia after several generations inherited this acquired character of refusing to ingest carmine, we are not told. These important details as well as others to which attention is called above, are not given in Metalnikow's paper, which is indeed only a preliminary paper. But until such details are made known, and until Metalnikow's experiments are described in detail, so that they may be verified, a discussion of our results which disagree in every essential particular, is futile.

THE BASIS OF SELECTION

A series of experiments will now be taken up which were designed for the purpose of ascertaining if possible upon what basis selection in Stentor is exercised. We have seen from the experiments up to this point that Euglenæ are eaten with more readiness than Phacus, and that carmine is more readily eaten than india ink, and that between living and dead organisms no selection seems to be exercised. What can be the basis of discrimination that gives such results? So far as we can tell there are only two possible methods by which Stentor can discriminate between two different substances; one is by a chemical sense ("tasting" and "smelling"), and the other by a tactual sense ("touching"). Does Stentor select a particle by "touching" it, or by "tasting it," or by both methods? (The psychological terminology is used here merely for convenience. By tasting and touching are meant reactions to chemicals and to contact and form, respectively.)

In the first experiments, organisms which are readily eaten by Stentor were altered as far as their chemical nature was concerned. Some were cooked, some soaked for a considerable time in alcohol, others in iodine, osmic acid, mercuric chloride, tannic acid, etc. Euglenæ, Trachelomonàs, and very small paramecia were the organisms employed for these purposes. After they had been treated with the chemicals mentioned, they were thoroughly washed and sucked up into pipettes. Hungry Stentors were isolated; then normal living organisms, and also those treated with the chemicals, were fed to the Stentors in mixed order, as was done in the first series of experiments. In no case were the chemically treated organisms rejected while the living were eaten. Some Stentors ingested all the organisms, and others rejected some of the living and some of the chemically treated, while the rest, including organisms of both kinds, were rejected.

It was found in previous experiments that Stentor could discriminate between Phacus triqueter and P. longicaudus, and between Trachelomonas hispida and T. volvocina, etc. In the experiment just preceding Stentor did not discriminate between living organisms and organisms killed with osmic acid, iodine, etc. Now it seems improbable that Stentor selects upon a chemical basis, since it is hard to understand how there could be a greater difference in "taste" between living Phacus triqueter and living P. longicaudus, where there was selection, than between living Phacus triqueter and specimens of P. triqueter killed with acids, iodine, etc., where there was no selection.

But to test this result further another set of experiments was performed in which the form and surface texture of food organisms was changed to a greater or less extent, but the chemical nature was left, as nearly as possible, in the same state as in the normal organism. Paramecia and Euglenæ were used for these purposes. By means of very small platinum knives these organisms were cut into halves or quarters, or even smaller pieces, and then fed in a stream with whole living organisms of the same species. There was no discrimination. The pieces were eaten as readily as the whole specimens. Many of these organisms were then mashed and minced into very fine fragments so that the original form and

surface texture was quite destroyed. This "jelly" was then sucked up and fed to Stentors together with normal living organisms of the same species. The whole specimens were eaten but the "jelly" was rejected. The Stentors bent away or reversed their cilia the moment the mashed paramecia or Euglenæ touched the disk or pouch, exactly as they do when a cloud of carmine ink, etc., is similarly caused to come in contact with the Stentor.

Small starch grains were then mixed with this jelly and diluted and fed to the Stentors. No starch grains were eaten. The Stentors still reversed their cilia when any of this mixture touched the disk.

Starch grains (potato and corn) were then fed in solutions of various strengths of sugar, beef juice, pork juice, pepsin, Liebig's Extract of Beef. The starch was invariably rejected. This same thing was tried with carmine and india ink, but the same results were got here as were derived from the controls; no more carmine or ink was ingested if pork or beef juice, or sugar, was present, than when these were absent.

Still other experiments were carried on in which the food organisms were soaked in various chemicals such as iodine, anilin dyes, alcoholic solutions of quinine, etc., and then only part of the superfluous chemical was washed away, so that when fed, a little of the iodine, quinine, etc., would be in solution and thus give the Euglenæ or paramecia quite a different "taste" from that which these organisms normally possess. These experiments are very difficult to carry out, owing to the fact that when iodine or quinine is just a little too strong, the Stentors contract the moment these substances touch the disk, and yet if one washes these chemically treated organisms a little longer one cannot be sure that any iodine or quinine remains to affect the Stentor before the organisms are swallowed. The experiments are also somewhat uncertain, for in the case where an organism is eaten, we cannot be sure whether the quinine, dye, or iodine, etc., had any effect on the Stentor at all. But the following are the results. When the food organisms were not too vigorously washed, the Stentors that were fed with them bent away or contracted the moment the stream from the pipette touched the disk. When the washing was carried a little further the organisms were ingested as were the living organisms.

Now if Stentor selects its food upon a chemical basis we should expect something like the following to occur. 1. Living organisms ought to call forth a different reaction from those cooked or chemically treated, since it seems evident that the "taste" of these two classes of food is very different. 2. When living organisms are fed we should expect the behavior to differ from what occurs when they are fed in sugar solution, etc. 3. We should not expect carmine or ink to be eaten. 4. Starch grains would probably be eaten if soaked in, and fed with, paramecium juice; and the minute fragments of paramecia would also probably be eaten if Stentor selected its food upon a chemical basis. But as a matter of fact the reverse is true in each case.

But let us see what Stentor would probably do if it selected its food upon a tactual basis. Under tactual stimuli all those of size, weight, form, and surface texture, and discrimination might be made upon any one or all of these factors.

It is clear from the outset that size plays little or no part in the selection, for all sizes of organisms from bacteria to paramecia are ingested. So we have only to consider weight, form, and surface texture.

Selection upon the basis of weight alone would result in rejecting all substances which differed in weight (specific gravity) from living organisms. This would explain the rejection of glass, sand, starch, etc., and the ingestion of carmine and ink. But it probably would not explain selection obtaining between Phacus and Euglena or between Euglena and Chilomonas, or between a rapidly moving organism like Halteria and any other particle.

Selection on the basis of form alone would also fail to explain all the results obtained in the experiments. For a Phacus differs more from a Euglena in form than from a starch grain, etc.

Nor would the factor of surface texture completely explain all the results obtained in the experiments.

It seems clear therefore that as far as my experiments go, no single quality such as weight, or form, etc., is decisive for setting off the Stentor's ingesting mechanism in all cases where discrimination occurs. It is probable that more than one factor serves as a basis for discrimination.

But whatever the basis is, it is constantly varying. The degree of hunger, as we have seen, has a marked influence upon selection. Hunger perhaps does not form part of the real basis of selection inasmuch as it only influences the degree of accuracy in discrimination. The same statement may be made as regards mere contact with food. As was shown in experiments described on page 119, the mere contact of paramecia with Stentor seems, like hunger, to have increased the degree of accuracy in discrimination.

The experiments then in this part show that Stentor probably selects its food upon a tactual and not upon a chemical basis. Further the experiments seem to indicate that more than one of the factors, weight, form, or surface texture, serve as a basis for discrimination.

THE SELECTIVE MECHANISM

We have seen that there are two mechansims by means of which discrimination is directly brought about. The ingesting mechanism is set into action by certain qualities in particles, when the Stentor is hungry. The absence of these qualities, or the presence of qualities which are objectionable to Stentor, call into action the rejecting mechanism. These two mechanisms, taken each by itself, are constantly varying with regard to the strength of stimulus required to set either into action. As the Stentor passes from a hungry to a satiated stage the ingesting apparatus is continually less and less easily set off. But with the rejecting apparatus the reverse is true. As the Stentor grows more and more satiated, constantly weaker stimuli set off the rejecting mechanism until finally all particles stimulate it into action.

These changes are due to the constantly varying physiological state, brought about by the constantly accumulating food in the body of Stentor. That is, the amount of food ingested regulates solely the ease with which the ingesting or the rejecting mechanisms are set off. But in some cases there is evidently a direct effect produced in the stimulus-receiving organ by the stimulus. Such cases are those experiments in which carmine and food particles were fed, sometimes mixed, and sometimes each substance

by itself. Some Stentors from the beginning of the experiment rejected carmine apparently only because food was also present. The rejecting and the ingesting mechansism may therefore be acted upon and their mode of action in consequence changed, both by the physiological state as determined by the amount of food in the animal, and by the condition of the stimulus-receiving apparatus as influenced by the amount of previous stimulation.

Whenever a particle possesses qualities that stimulate to a greater or less extent both the ingesting and the rejecting mechanism, the action of the cilia in the pouch and funnel are not thoroughly coördinated either for ingesting or for rejecting, and as a consequence loops are formed in the path of the particle. It seems from the experiments that the stimuli from the objectionable features of a particle are summated more rapidly than the stimuli from the desirable features; for 80 per cent of all the particles that had loops in their paths were finally rejected. We also find that a much larger number of loops occur when the Stentor is beginning to be replete than when hungry or when almost satiated. At the stage when the animal is just beginning to be replete, stimuli of medium intensity are required to set off either the ingesting or the rejecting apparatus. The stimuli are more nearly of equal intensity, or in other words, the mechanisms are set off more nearly with equal readiness at this stage than at any other. This is probably the reason for the larger number of loops at this stage.

Selection between food and indigestible particles in Stentor is an almost perfect adaptation. With very few exceptions only particles (organisms) of food value are ingested. Indigestible particles of many sorts which have for thousands of generations not come in contact with Stentor are nevertheless rejected with accuracy.

SUMMARY

1 Stentor cæruleus exercises a selection among the particles that are brought to its food pouch by the ciliary current. The selection is brought about by changes in the beat of the cilia of the pouch and funnel. Certain particles are rejected by a localized

reversal of the cilia; others are carried to the mouth and ingested. Of two particles within the funnel at the same time one may be thus rejected while the other is ingested. Selection thus occurs not only from among particles reaching the pouch successively, but from a large number reaching the pouch at once.

2 Stentor discriminates very accurately between organisms (Phacus, Euglena, etc.) and indigestible particles (carmine, glass, sulphur, starch, etc.), ingesting the former and rejecting the latter.

3 Stentor discriminates between different kinds of organisms, eating some (Euglena, Phacus triqueter) with great readiness, while others (Trachelomonas hispida, Phacus longicaudus) are rarely ingested.

4 States of hunger and satiety, and intermediate conditions, are shown to exist in Stentor by differences in the behavior toward food. The animal discriminates more perfectly (i. e., more restrictively), when almost satiated than when very hungry. When very hungry it may ingest many indigestible particles (carmine, india ink, etc.)

5 The amount of a given substance ingested depends upon what other substances are present. Stentor in water containing indigestible particles, such as carmine, may ingest much of the latter; if the water contains in addition many organisms fit for food, very little of the indigestible matter is ingested.

6 It was not found that Stentor and paramecium become lastingly "educated" to reject certain sorts of food that they have formerly taken. Such changes as occur in selection seem to be mainly matters of hunger and satiety.

7 Stentor selects its food upon a tactual basis and apparently not upon a chemical one. That is, Stentor reacts in selecting food, to physical properties only or chiefly, and not to chemical properties.

8 Stentor in a condition of satiety differs in many respects from Stentor in a condition of hunger. In satiety we find the following conditions: *a*. Extension is always submaximal. *b*. The aboral side is more strongly convex than the oral side. *c*. There is a marked decrease in the activity of the membranellæ. *d*. Stentors respond much more readily to mechanical stimulation

at the disk. *e.* If the stimuli are submaximal, contraction is often only partly accomplished.

9 The amount of food in the body of the Stentor at any given moment is not an accurate register of the degree of satiety; the latter depends upon what recent stimuli have been received, as well as on the amount of food in the body.

REFERENCES

Bütschli, O. '87–'89—Protozoa. Vol. i of Bronn's Thierreich. Leipzig.

Ehrenberg, C. G., '38—Die Infusionsthiere als vollkommene Organismen. Leipzig.

Entz, Géla '88—Studien über Protisten. Aufträge der königl. Ung. Naturw. Ges. Budapest.

Hodge, C. F., and Aikins, H. A. '93—The Daily Life of a Protozoan. Amer. Journ. of Psychology, vol. vi.

Jennings, H. S. '02—Studies on Reactions to Stimuli in Unicellular Organisms. viii. Amer. Journ. of Psychology, vol. vi.

Meissner, M. '88—Beiträge zur Ernährungsphysiologie der Protozoen. Zeitschr. f. wissensch. Zool. Bd. 46.

Metalnikow, S. '07—Uber die Ernährung der Infusorien und deren Fähigikeit ihre Nahrung zu wählen. Travaux de la Societe Imp. des. Naturalistes de St. Petersbourg. Bd. 38, Lief. 1, No. 4.

Stein, F. v. '67—Der Organismus der Infusionsthiere. Bd. II. Leipzig.

Verworn, M. '89—Psychophysiologische Protistenstudien. Jena.